

Listing of the Claims

The following listing of the claims, and the amendments contained herein, replace all prior versions and amendments of the claims.

Claims 1-7. Canceled.

Claim 8. (Amended) A method for the analysis of the methylation status of one or more CpG dinucleotides within a nucleic acid sample, comprising:

a. in the nucleic acid sample, converting cytosine bases that are unmethylated at the 5-position by treatment with a converting agent to uracil or another base which is dissimilar to cytosine in terms of base pairing behavior;

b. amplifying one or more nucleic acids of the treated nucleic acid in a polymerase enzyme reaction by means of at least ~~two~~ three primer oligonucleotide pairs, wherein one primer pair does not contain a CpG dinucleotide and does not contain a TpG dinucleotide and amplifies a reference sequence and the other primer pairs are methylation specific primers, and further wherein the amplicates formed from each species of primer pairs differ respectively in at least one of length, sequence, and a detectable label selected from [a] the group consisting of fluorescence labels, mass labels, and radioactive labels;

c. detecting the amplicates formed from the primer pairs;

d. measuring the amounts of the amplicates formed from each primer pair; and

e. determining the degree of methylation at each analyzed CpG position from ratios of amplicates formed from each of said methylation specific primers relative to amplicates formed from said primer pair that amplifies said reference sequence.

9. (Previously presented) The method of claim 8, wherein the converting agent is bisulfite or a compound thereof.

10. (Previously presented) The method of claim 8, wherein the detection of amplicates is carried out by one of mass spectrometry and a real time technique.

11. (Previously presented) The method of claim 10, wherein detection by mass spectrometry is carried out by means of matrix assisted laser desorption/ionization mass spectrometry (MALDI) or electron spray mass spectrometry (EST).

Claims 12-14. Canceled.

15. (Amended) The method of claim [13] 8, wherein the primer pairs that do not amplify the reference sequence include one or more of CpG, TpG, and CpA dinucleotides.

16. (Amended) The method of claim [13] 8, wherein the amplicate synthesized from each primer pair is compared to the amplicate from the other primers and to the amount of amplicate from the reference primer.

17. (Amended) The method according to claim [13] 8, wherein determining the degree of methylation is carried out by determining the amount of each amplicate from each primer pair relative to the amount of amplicate formed from the reference primer pair.

18. (Amended) The method of claim [13] 8, wherein the amplicates are modified ~~in such a manner that they become similar to peptides~~ by alkylation and optionally charge tagging to enhance sensitivity of detecting said amplicates formed.

Claims 19-24. Canceled.

Claim 25. (Withdrawn) A plurality of oligonucleotide primer pairs for the determination of the degree of methylation at one or more CpG positions in a nucleic acid sample, wherein the oligonucleotide primer pairs are capable of distinguishing between methylated and non-methylated nucleic acid in the sample after modification by bisulfite treatment, and further wherein a first primer pair hybridizes preferentially to a modified nucleic acid that was methylated in the nucleic acid sample in the sequence the primer is hybridizing to, and a second primer pair binds preferentially to modified nucleic acid that was methylated in the nucleic acid sample in the sequence the primer is hybridizing to.

Claim 26. (Withdrawn) The primer pairs of claim 25, including at least one reference primer pair that is methylation insensitive.

Claim 27. (Withdrawn) The primer pairs of claim 25, wherein the amplicates synthesized from all species of primers are comparable to each other and differ according to at least one of length, sequence and detectable label and are thereby differentially detectable and quantifiable.

Claim 28. (Withdrawn) The primers of claim 25, wherein the amount of amplificate from each primer pair is compared to the amount of amplificate synthesized from the reference primer pair.

Claim 29. (Withdrawn) A kit providing for analysis of the methylation status of one or more CpG dinucleotides within a nucleic acid sample, comprising apparatus including a plurality of segments, including at least a first segment that contains an agent for converting unmethylated cytosines to another nucleotide base within the nucleic acid sample, a second segment that contains at least two oligonucleotide primer pairs that hybridize with a target polynucleotide sequence and amplify CpG containing nucleic acid, one of which primer pairs hybridizes preferentially to converted nucleic acid that was methylated in the original sample in the sequence the primer is hybridizing to, and a second of which pairs hybridizes preferentially to converted nucleic acid that was unmethylated in the original sample in the sequence the primer is hybridizing to, and instructions for carrying out the conversion and amplification, for detecting the amplicates formed in the polymerase reaction in a quantifiable manner and for determining the degree of methylation in at least one selected segment of the nucleic acid sample.

Claim 30. (Withdrawn) The kit of claim 29, further comprising at least one primer pair that either 1) amplifies a non-methylated sequence that acts as a reference sequence, or 2) is methylation insensitive.